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TITLE: The Effects of Antioxidants and Experience on the Development of Age Dependent Cognitive Dysfunction and Neuropathology in Canines

PRINCIPAL INVESTIGATOR: Bruce A. Muggenburg, DVM, Ph.D.
Elizabeth Head, Ph.D.

CONTRACTING ORGANIZATION: Lovelace Biomedical and Environmental Research Institute
Albuquerque, New Mexico 87185

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Bruce A. Muggenburg, DVM, Ph.D.
Elizabeth Head, PhD

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Lovelace Biomedical and Environmental Research Institute

Albuquerque, New Mexico 87185
e-mail:
bmuggenb@lrri.org

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13. ABSTRACT (Maximum 200 words)

The purpose of this research is to evaluate a diet high in antioxidants, environmental enrichment, and the combination of both treatments as interventions to prevent or slow down age-related cognitive decline and brain pathology in dogs. The study is a 3-year longitudinal design. During the first year, the dogs underwent baseline screening of cognitive function and general health, plus a magnetic resonance image (MRI) of the brain. The MRIs are being used to obtain *in vivo* measures of brain and cerebrovascular function. After completion of the baseline measurements, the dietary and environmental interventions were started. During the second year, the one-year re-evaluations were performed and showed that the antioxidant diet increased the peripheral blood measures of antioxidants, general health was unchanged, and visuo-spatial and learning skills were improved over controls. MR measurements indicate that the size of the brain ventricles are increasing with age, but this may be reduced in the dogs receiving the combined treatment. Through additional collaborations, several new endpoints have been optimized to evaluate peripheral and central measures of neuropathology and oxidative damage. At the end of the study, detailed histological analysis of brain tissue will be correlated with cognitive function and MR measures of brain anatomy and cerebrovascular function to establish the effectiveness of the treatments on delaying or preventing the development of age-related neuropathologies.

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I. INTRODUCTION

The purpose of the current research project is to determine the effects of both antioxidants and environmental enrichment on age-dependent cognitive decline in a 3-year longitudinal design using beagle dogs. Dogs have undergone baseline screening of cognitive function and a general health evaluation including clinical pathology and physical examinations. Magnetic resonance imaging (MRI) scans are being used to obtain in vivo measures of brain and cerebrovascular function. Each dog is in one of four treatment groups, which are counterbalanced with respect to cognitive ability, sex, and age: (1) control group, (2) enriched environment, (3) antioxidant diet, and (4) combined dietary and environmental enrichment. A broad spectrum of antioxidants is being added for dietary enrichment. The environmental enrichment condition consists of additional cognitive experience and enriched sensory environment. Cognitive function, physical health, and brain MRIs are being monitored yearly to establish ongoing effects of the treatment. At the end of the study, detailed histological analysis of brain tissue will be correlated with cognitive function and MRI measures of brain atrophy and cerebrovascular function to establish the effectiveness of the treatments on delaying or preventing the development of age-dependent neuropathologies.

II. BODY OF THE REPORT

In Year 2, we proposed to have completed the first year of dietary and environmental enrichment in the study and to have begun the first year's annual re-evaluation of cognitive ability in 24 of the dogs that originated from the LRRI colony. The 24 dogs that originated from another dog colony have been on treatment for 6 months. Each measure obtained in the second year is described next.

A. Landmark Testing

A sample of 12 aged beagle dogs and 13 young dogs (at another site used for comparison) were given a battery of baseline learning and memory tasks prior to placement into either a control or antioxidant diet group. As the graph in Figure 1 illustrates for aged animals, dogs were equally matched with respect to learning (discrimination reversal) and memory (object recognition and spatial nonmatching). A t-test was used to compare the two groups of dogs on their baseline learning of the discrimination reversal learning, object recognition memory, and spatial nonmatching tasks. The results, which are revealed in Figure 1, were not significant ($p < 0.6229$). Thus, dogs were equally matched on the basis of cognition prior to diet intervention.

Approximately 1 month after starting the antioxidant diet, the first learning problem given to dogs was a landmark discrimination, which is a test of spatial attention (Milgrim *et al.*, 1999). Landmark discrimination learning requires subjects to select a particular object based on proximity to an object. The initial learning, however, is based on the dogs' ability to learn an object discrimination task. We have previously found that the effects of age on discrimination learning depends on task difficulty, and our preliminary evidence indicates that landmark discrimination learning is markedly impaired in aged dogs.

When old animals on the antioxidant and control diets were compared on the landmark discrimination learning task, there was a highly significant difference between the groups ($t(10) = 2.757$, $p < 0.02$). The results of this study are summarized in Figure 2. Animals on the antioxidant diet acquired the task with fewer errors than did the animals on the control diet. Whereas all six animals on the antioxidant diet met the learning criterion within 40 sessions, only three of the six animals on the control diet met the learning criterion. In addition, the three dogs that solved the problem committed more errors than dogs receiving the antioxidant diet. On the other hand, no statistically significant differences were found in young dogs given an antioxidant diet as compared to a control diet.

B. Oddity Testing

Dogs in the enriched environment group, after completing landmark discrimination learning, were tested on an oddity task. This task involves presenting dogs with three objects covering all three food wells. Two of these objects were identical, and one was different. To obtain a food reward, dogs had to select the odd object. Figure 3 shows that dogs on the antioxidant diet learned this task with significantly fewer errors than dogs fed the control diet ($t(8) = 4.327$, $p < 0.003$ for all four oddity test scores combined).

C. Blood Biochemistry

Blood biochemistry has been completed for LRRI dogs at the 6-month timepoint. All values fell within normal ranges with the expected variability (see Appendix A).

D. Vitamin E

Serum vitamin E levels were measured in the second group of dogs after 3 months of eating the antioxidant diet, and the results are illustrated in Figure 4. Whereas vitamin E levels were not different among the four treatment groups prior to intervention ($F(3,21) = 1.124$,

p = n.s.), dogs fed the antioxidant diet showed a significant increase in vitamin E levels ($F(3,21) = 9.362$, $p < 0.0001$).

E. Optimization of Oxidative Damage Endpoint Measures

Our study proposal outlined a series of oxidative damage endpoint measures to be used during neuroanatomical studies. In addition to the initially proposed immunolabeling for nitrotyrosine, we have identified and optimized new markers that will be used both to monitor treatment efficacy using peripheral samples and for later anatomical studies. We have conducted pilot studies from archived samples for measuring lipid peroxidation (malondialdehyde or MDA), and we have developed a new antibody in-house to detect oxidatively modified amyloid (oxidized-Aβ).

To measure the extent of lipid peroxidation, a marker for oxidative damage to lipids, MDA formation was measured in collaboration with Drs. Jiankang Liu and Bruce Ames at the University of California, Berkeley. Serum, cerebrospinal fluid (CSF), and brain samples (prefrontal cortex) were taken from a sample of 20 archived cases that had been behaviorally characterized. As shown in Figure 5, MDA levels in the brain were significantly elevated with aging ($F(1,17) = 12.144$, $p < 0.003$) ($r = 0.657$, $p < 0.003$). MDA levels were a significant predictor of Aβ deposition in the prefrontal cortex ($r = 0.70$, $p < 0.001$, $n = 13$), even when the effects of age were not used ($r = 0.5041$, $p < 0.039$, $n = 13$). MDA levels were significantly correlated with cognitive function; higher error scores on the size discrimination task were associated with increased levels of MDA ($r = 0.534$, $p < 0.04$). Serum levels of MDA predicted brain levels of MDA ($r = 0.494$, $p < 0.037$), and thus serum assays may serve as a measure of brain oxidative stress.

A new antibody, anti-oxidized-Aβ, was used to characterize the distribution of oxidatively modified Aβ in the prefrontal cortex of a young and an old dog. Oxidized-Aβ was present within a subset of diffuse plaques as illustrated in Figure 6. Thus, we will use this antibody to detect oxidatively modified Aβ to determine if treatment effects reduce the extent of this form of Aβ deposition.

F. MRI Retest

Year 1 evaluation MRIs have been conducted on all 48 dogs. All dogs tolerated the procedures well and suffered no adverse side effects. Significant correlations between baseline and Year 1 measures indicate good standardization of procedures across the year of

study. Figure 7 illustrates that over the year, dogs in the control ($t(9) = 6.229$, $p < 0.0001$), enriched environment/control diet ($t(9) = 7.919$, $p < 0.0001$), and control/antioxidant diet ($t(9) = 3.999$, $p < 0.003$) showed significant increases in ventricular volume. This age effect was reduced in the group of dogs receiving the combined treatment ($t(11) = 1.992$, $p < 0.072$). MRI scans from baseline and Year 1 from a representative dog are shown in Figure 7 and illustrate increasing ventricle size with age. Dr. Lydia Su at the University of California as per our protocol is conducting the ongoing anatomical and cerebrovascular analyses.

III. KEY RESEARCH ACCOMPLISHMENTS FOR YEAR 2

- The first year of dietary and environmental enrichment has been completed.
- Three-month measures of vitamin E indicate that the diet continues to improve peripheral measures of antioxidant status.
- The 6-month blood biochemistry measures indicate that the antioxidant diet has had no adverse events.
- The antioxidant diet improves visuo-spatial skills in aged dogs.
- The antioxidant diet improves learning on a new task – the oddity task.
- Several new endpoints have been optimized to evaluate both peripheral and central measures of neuropathology and oxidative damage.
- MRI measures indicate that ventricular size is increasing with age, but this may be reduced in dogs receiving the combined treatment.

IV. REPORTABLE OUTCOMES

We have published several abstracts for the Annual Meeting of the Society for Neuroscience discussing the results of the cognitive studies completed, and these abstracts are included as Appendices B–F.

B. Oxidative Stress, β-Amyloid and Cognitive Function in the Aged Canine Brain. E. Head, J. Liu, B. N. Ames, B. A. Muggenburg, N. W. Milgram, and C. W. Cotman

C. Visual Object Recognition Memory in Beagle Dogs: Effect of Processing Time and Age. C. J. Ikeda-Douglas, H. Callahan, E. Head, C. Cotman, J. Araujo, A. Chan, J. Estrada, B. Muggenberg, S. Zicker, N. W. Milgram, and W. Mackay

D. Landmark Discrimination Learning in Aged Dogs is Improved by Treatment with an Antioxidant Enriched Diet. N. W. Milgram, J. Estrada, C. Ikeda-Douglas, J. Castillo, E. Head, C. W. Cotman, H. Murphey, D. Holowachuk, B. Muggenberg, and S. Zicker

E. Dr. Milgram has been invited to present this abstract in a press conference being organized by the Society for Neuroscience in November 2000. Appendix E provides a copy of the email that we received and indicates the interest from the Society in this study.

F. A Novel Spatial Learning and Memory Task: The 3-Component Delayed Non-Matching-to-Position Task. P. Nippak, A. Chan, B. Adams, C. Ikeda-Douglas, E. Head, C. Cotman, B. Muggenberg, S. Zicker and N. W. Milgram

In addition, three manuscripts listed below are in preparation, and we hope to have these submitted by January 2001.

1. Milgram, N. W. *et al.*, Oddity Discrimination Learning as a Function of Age and Antioxidant Diet in Beagle Dogs.

2. Chan, A. *et al.*, Cognitive Test Experience Can Partially Alleviate Age-Dependent Visuospatial Deficits in Beagle Dogs.

3. Head, E. *et al.*, Oxidative Stress, β-Amyloid and Cognitive Function in the Aged Canine Brain.

V. CONCLUSIONS

The goals for Year 2 were to complete 1 year of intervention in 24 LRRI dogs using four treatment groups. Twelve of these animals are receiving environmental enrichment and physical exercise in addition to further learning experiences. Twelve of these dogs, in turn, are also receiving the antioxidant diet, and we have obtained the first information regarding the effects of antioxidants on learning ability. Dogs were tested for landmark discrimination and oddity learning. All dogs have received 3-month vitamin E measures, 6-month blood biochemistry measures, and health examinations. We now have exciting data indicating that an antioxidant diet can increase peripheral levels of vitamin E and improve learning in aged animals. In our study outline, we also indicated that we would obtain the first annual MRI scans for comparison with baseline measures. The most striking effect is the consistency across MRI scan images. Further, there appears to be a significant reduction in ventricular widening in dogs receiving the combined treatment of antioxidant diet and environmental enrichment. As of this report, the study is on schedule, and valuable information regarding the potential of a diet rich in antioxidants in improving learning ability in aged dogs has been obtained.

VI. REFERENCES

Milgram, N. W., B. Adams, H. Callahan, E. Head, W. Mackay, C. Thirlwell, and C. W. Cotman. Landmark discrimination learning in the dog. *Learning & Memory 6*: 54-61 (1999).

VII. FIGURES

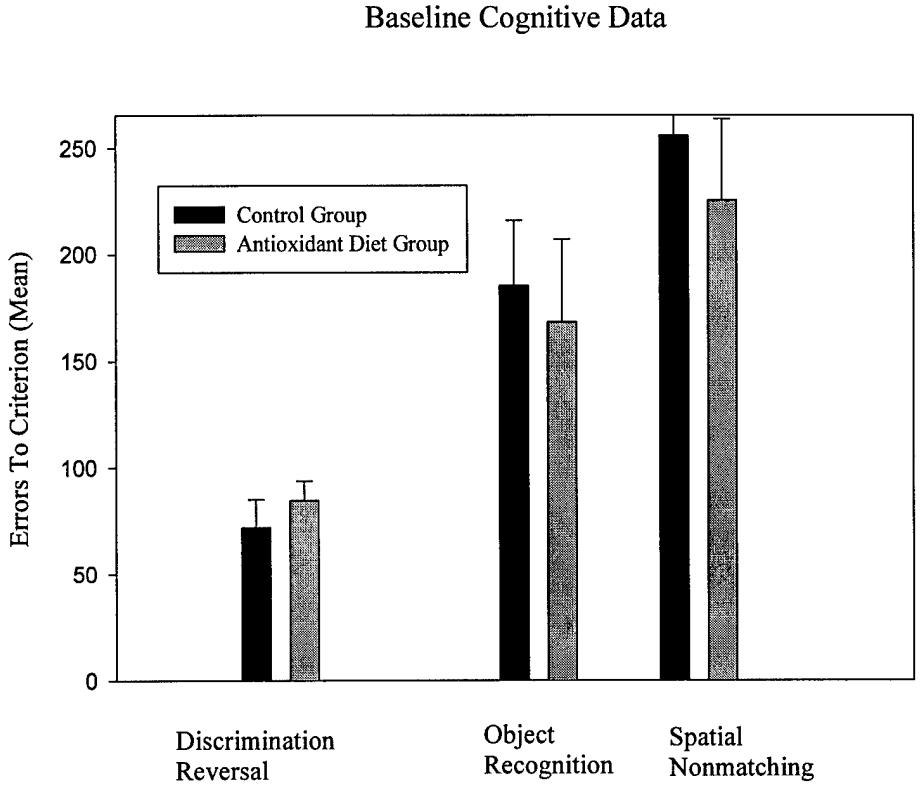

Figure 1. The two groups of subjects did not differ in baseline acquisition of a discrimination reversal learning task, an object recognition task, and spatial memory task. Error bars illustrated standard error of the mean.

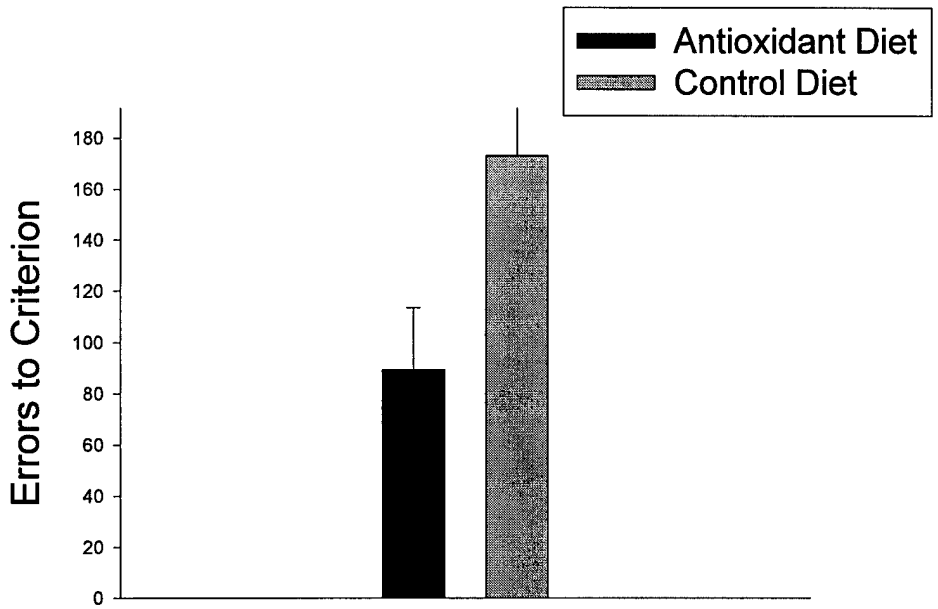

Figure 2. Acquisition of a complex discrimination learning task in animals on a control diet as compared to animals fed a diet rich in antioxidants. Error bars represent standard error of the mean.

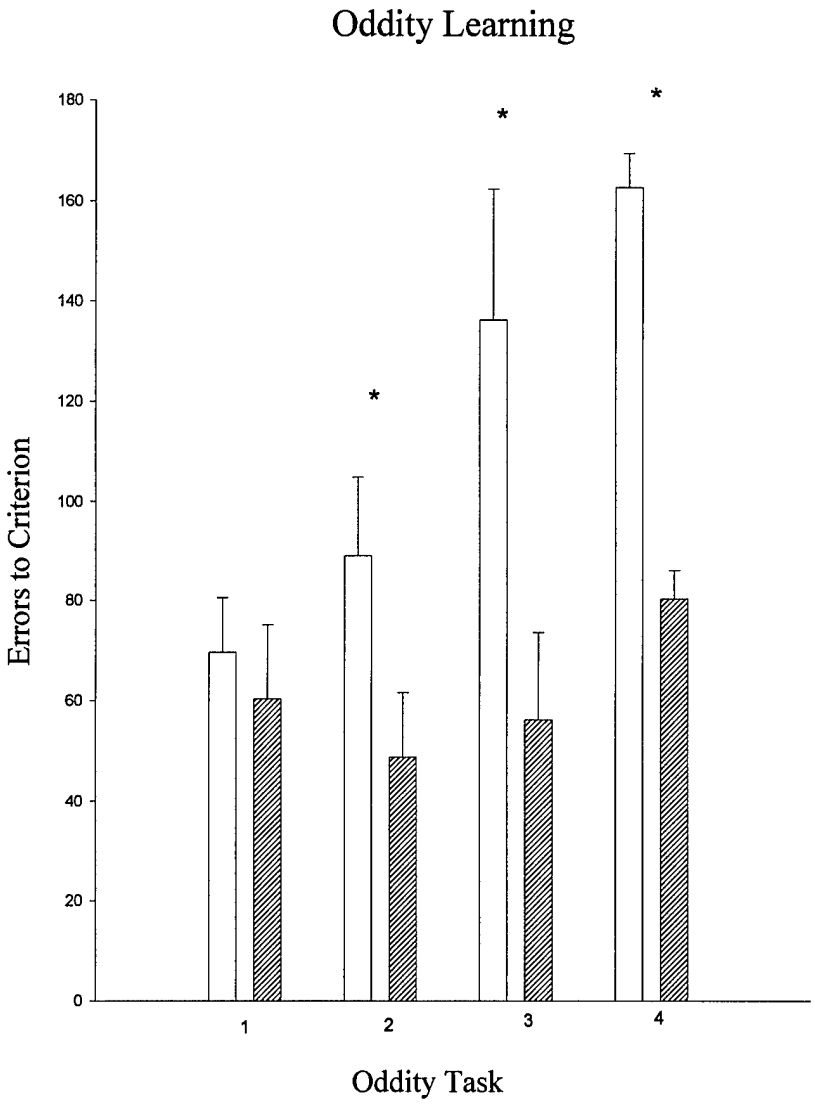

Figure 3. Oddity learning is significantly improved in dogs fed a diet rich in antioxidants. Dogs were given four oddity problems with different sets of objects used for each test (as indicated on the x axis). Bars represent the mean error scores shown for the control (open bars) or antioxidant diet (hatched bars) groups. Error bars represent standard errors of the mean. Asterisks indicate significantly different at the $p < 0.05$ level.

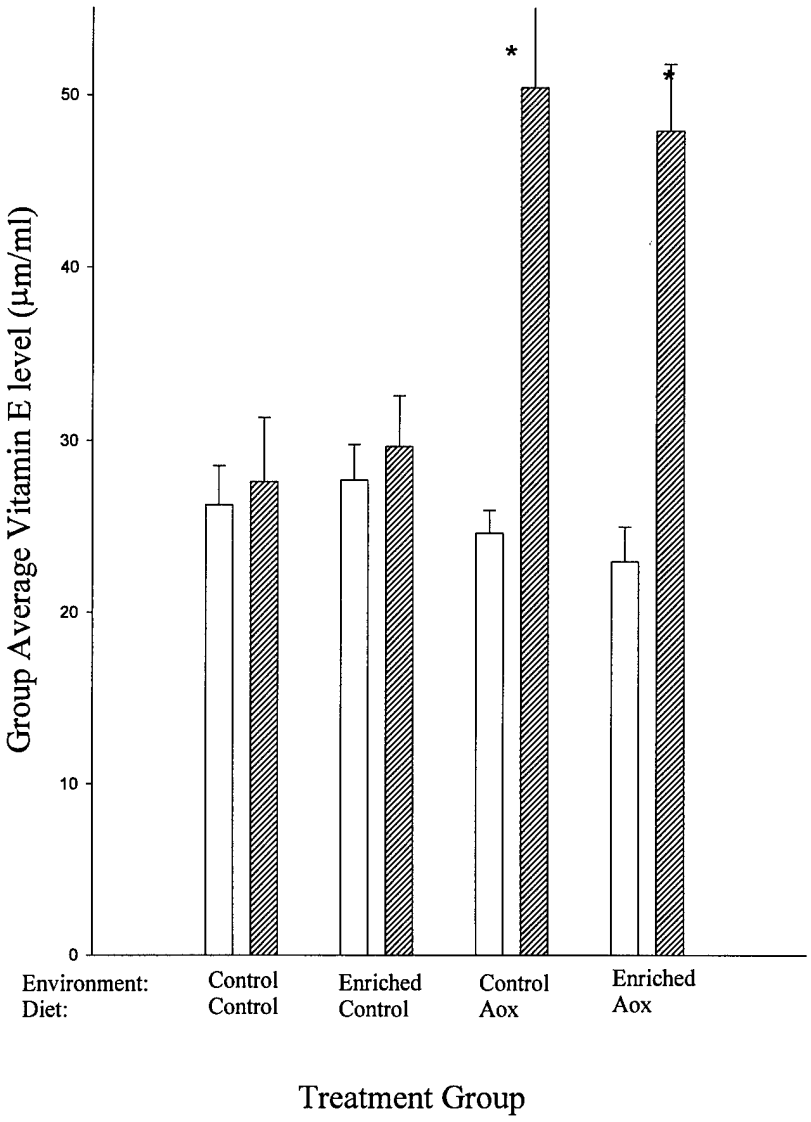

Figure 4. Average serum vitamin E levels are plotted as a function of treatment group. The open bars indicate baseline measures, and the hatched bars are measures obtained after 3 months on the diet rich in antioxidants (Aox). Error bars represent errors of the mean. Asterisks indicate that 3-month levels of vitamin E are significantly higher than baseline levels ($p < 0.05$).

Figure 5. The extent of lipid peroxidation (malondialdehyde), a measure of oxidative damage, increases as a function of age in the brains of dogs. These were samples from archived tissues stored at the Institute for Brain Aging and Dementia at University of California, Irvine.

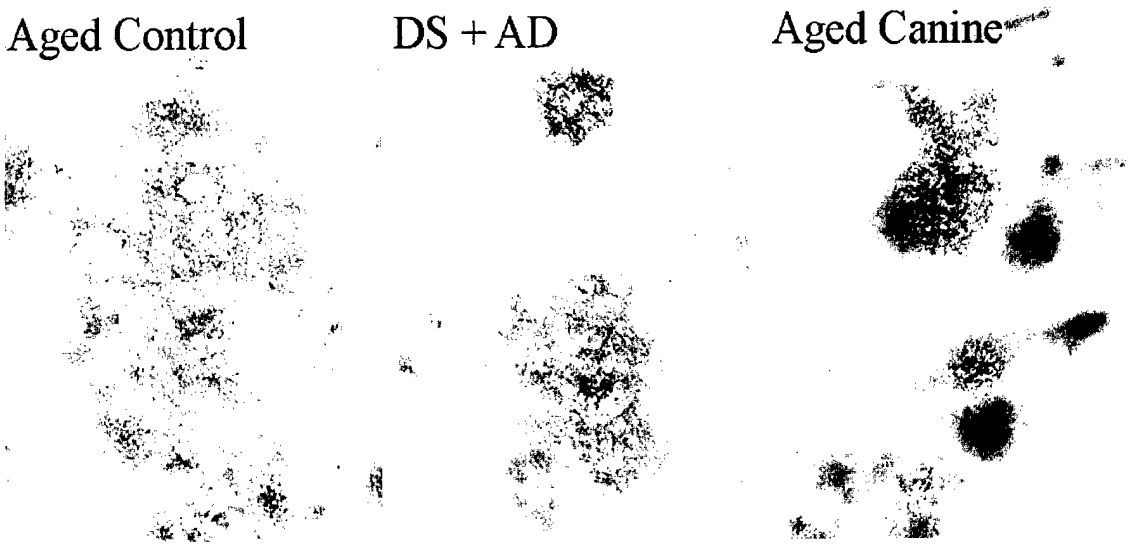

Figure 6. A new marker for oxidatively modified beta-amyloid was used for immunocytochemistry experiments in samples from the prefrontal cortex. Oxidatively modified amyloid is present within a subset of diffuse deposits in an aged canine (right panel) and appears strikingly similar to immunostaining in the aged human brain (left panel) and in the Alzheimer's disease brain (middle panel). Canine brain samples were obtained from archives maintained at the Institute for Brain Aging and Dementia at University of California, Irvine. Magnification is at 50 microns.

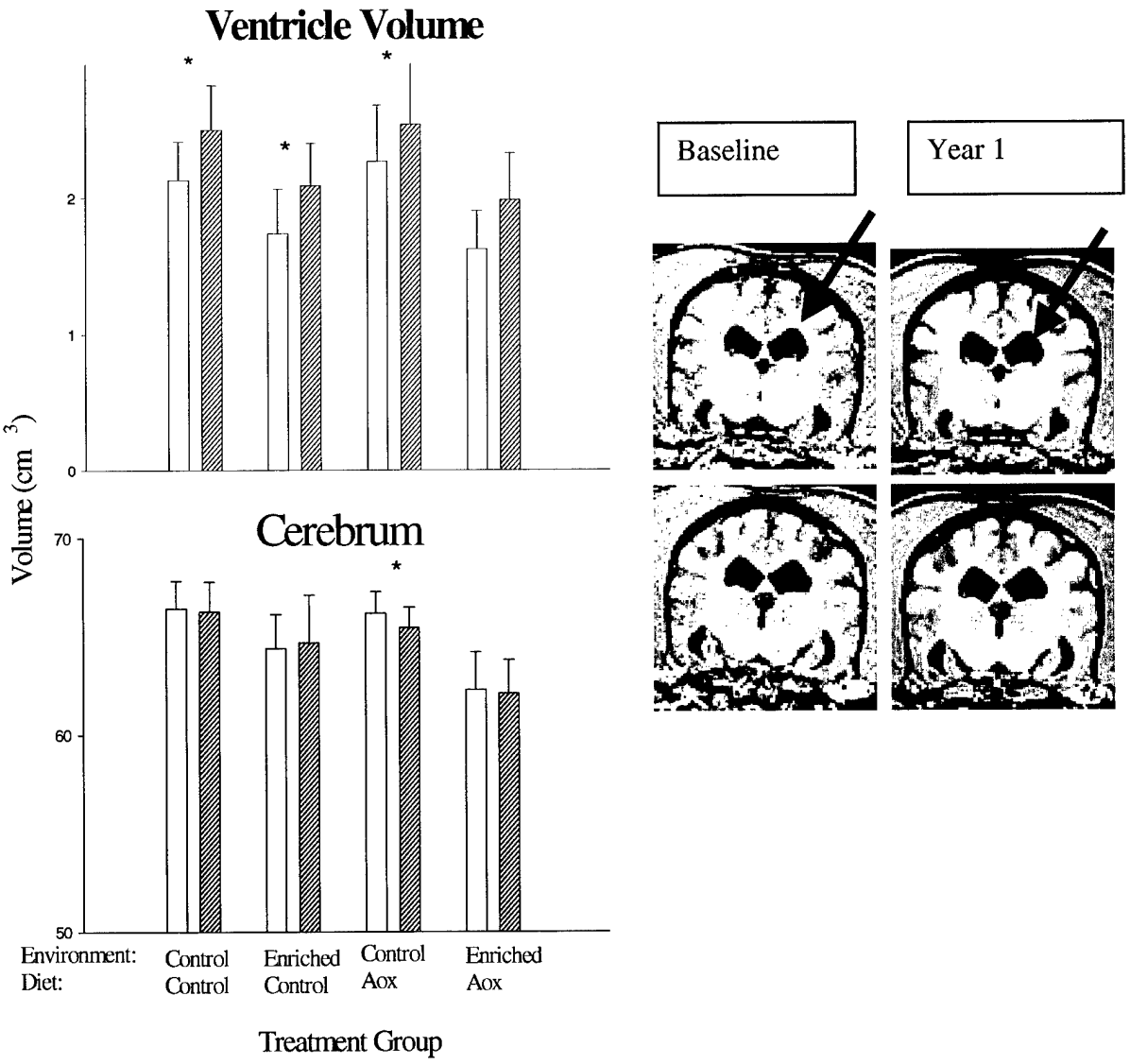

Figure 7. Baseline (open bar) and Year-1 (hatched bars) volumes are plotted as a function of treatment group for two variables. Asterisks indicate significant differences at the $p < 0.05$ level. Aox = antioxidant. Two sections from one aged dog on study are provided to illustrate that changes in ventricular volume from baseline to year one are subtle and thus require the finer volumetric analyses.

VIII. APPENDICES

Appendix A. Raw Data. The 1-Year Blood Biochemistry Values from Individual Dogs in Each Treatment Group

	Control/Control					
Animal ID	**1510A**	**1494D**	**B2150**	**1508U**	**1543S**	**1521S**
Date	3/21/00	2/16/00	1/25/00	2/16/00	1/25/00	2/16/00
Tests Performed						
AST (SGOT)	31	29	36	32	19	28
ALT (SGOT)	45	50	49	26	63	30
T. BILIRUBIN	0.1	0.1	0.1	0.2	0.2	0.2
ALK PHOS	97	78	85	53	118	78
GGT	2	4	3	1	9	2
TOTAL PROTEIN	6.7	6.6	6.4	6.1	7.0	5.8
ALBUMIN	3.5	3.1	3.5	3.4	3.3	2.6
GLOBULIN	3.2	3.5	2.9	2.7	3.7	3.2
A/G RATIO	1.1	0.9	1.2	1.3	0.9	0.8
CHOLESTEROL	252	152	201	141	183	272
BUN	13	12	14	11	9	12
CREATININE	0.8	0.5	0.5	0.5	0.5	0.8
BUN/CREAT	16	24	28	22	18	15
PHOSPORUS	3.7	3.2	2.9	3.3	3.7	4.2
CALCIUM	9.5	9.6	9.7	9.9	9.5	8.0
CA/PO4	2.6	3.0	3.3	3.0	2.6	1.9
GLUCOSE	83	79	98	90	77	86
AMYLASE	897	1106	620	636	606	638
LIPASE	77	364	338	350	410	114
SODIUM	142	146	144	145	143	154
POTASSIUM	4.1	4.7	4.9	4.3	4.7	4.8
NA/K RATIO	35	31	29	34	30	32
CHLORIDE	105	109	108	111	107	114
CPK	283	176	201	166	83	180
TRIGLYCERIDE	51	196	106	41	126	72
OSMOLALITY- CALC	293	301	298	299	293	317
CORRECTED CA	.	10.0	.	10.0	9.7	8.9
MAGNESUIM	2.2	1.4	.	2.0	.	1.1
WBC	7.2	9.6	6.6	10.6	10.4	6.6
RBC	6.6	7.6	6.4	8.1	7.1	6.6
HGB	15.6	16.4	13.9	16.6	16.3	14.7
PCV	47	51	42	51	49	45
MCV	72	67	67	64	70	68
MCH	23.8	21.6	21.9	20.7	22.9	22.4
MCHC	33	32	33	33	33	33
NEUTROPHILS	73	67	74	73	69	74
ABSOLUTE	5256	6432	4884	7738	7176	4884
LYMPHOCYTES	18	20	17	12	22	17
ABSOLUTE	1296	1920	1122	1272	2288	1122
MONOCYTES	8	12	9	7	8	9
ABSOLUTE	576	1152	594	742	832	594
EOSINOPHILS	.	.	.	8	.	.
ABSOLUTE	.	.	.	848	.	.
BASOPHILS	1	1	.	.	1	.
ABSOLUTE	72	96	.	.	104	.
PLATELET EST.	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate

Animal ID	1510A	1494D	B2150	1508U	1543S	1521S
Date	3/21/00	2/16/00	1/25/00	2/16/00	1/25/00	2/16/00
T3 (RIA)	134	112	102	78	73	144
T4 (RIA)	1.36	1.25	0.75	1.35	0.9	1.85
FREE T4 (RIA)	1.12	1.37	0.80	1.42	0.7	1.36
T3AA	0.9	0.8	1.1	.	1.0	0.9
T4AA	0.8	1.0	1.1	1.1	1.2	1.0
FREE T3	4.0	3.9	2.2	2.2	1.7	3.5
TSH	<0.1	0.25	0.90	0.2	3.62	0.25
QUANT. PLATELET	389	362	464	332	.	491
ANISOCYTOSIS	Slight	Slight	Slight	Slight	Slight	Slight

	Enriched/Control					
Animal ID	**1518D**	**1506B**	**1492B**	**1542S**	**1529S**	**1523U**
Date	1/25/00	2/16/00		1/25/00	1/25/00	2/16/00
Tests Performed						
AST (SGOT)	38	24	dead	27	28	22
ALT (SGOT)	53	35		26	52	22
T. BILIRUBIN	0.1	0.1		0.1	0.2	0.2
ALK PHOS	140	331		239	87	82
GGT	6	3		6	7	1
TOTAL PROTEIN	6.7	6.5		6.0	6.1	6.4
ALBUMIN	3.0	3.1		2.9	3.3	3.2
GLOBULIN	3.7	3.4		3.1	2.8	3.2
A/G RATIO	0.8	0.9		0.9	1.2	1.0
CHOLESTEROL	164	193		284	188	187
BUN	16	9		7	13	19
CREATININE	0.5	0.5		0.5	0.6	0.5
BUN/CREAT	32	18		14	22	38
PHOSPORUS	3.7	4.9		3.8	3.8	5.5
CALCIUM	9.2	10.1		8.7	9.4	9.8
CA/PO4	2.5	2.1		2.3	2.5	1.8
GLUCOSE	94	90		91	94	90
AMYLASE	1108	734		774	518	785
LIPASE	151	290		576	609	296
SODIUM	141	145		143	141	145
POTASSIUM	4.4	4.6		4.4	4.2	5.1
NA/K RATIO	32	32		33	34	28
CHLORIDE	108	110		109	106	111
CPK	176	165		104	117	140
TRIGLYCERIDE	88	115		187	59	74
OSMOLALITY- CALC	293	298		294	292	302
CORRECTED CA	9.7	10.5		9.3	9.6	10.1
MAGNESUIM	.	2.0		.	.	2.4
WBC	6.5	10.1		11.2	6.0	7.7
RBC	6.7	6.5		7.3	7.2	7.2
HGB	14.7	14.0		16.9	16.6	16.1
PCV	46	44		52	50	49
MCV	68	68		71	69	69
MCH	22.0	21.6		23.2	23.0	22.4
MCHC	32	32		33	33	33
NEUTROPHILS	71	71		77	77	69
ABSOLUTE	4615	7171		8624	4620	5313
LYMPHOCYTES	21	21		17	16	20
ABSOLUTE	1365	2121		1904	960	1540
MONOCYTES	6	8		6	5	11
ABSOLUTE	390	808		672	300	847
EOSINOPHILS	2	.		.	2	.
ABSOLUTE	130	.		.	120	.
BASOPHILS	.	.		.	.	.
ABSOLUTE	.	.		.	.	.
PLATELET EST.	Adequate	Adequate		Adequate	Adequate	Adequate

Animal ID	1518D	1506B	1492B	1542S	1529S	1523U
Date	1/25/00	2/16/00		1/25/00	1/25/00	2/16/00
T3 (RIA)	110	132		121	122	91
T4 (RIA)	1.75	1.50		1.54	2.05	1.22
FREE T4 (RIA)	1.05	1.45		1.19	1.34	1.48
T3AA	1.0	0.8		1.2	1.1	0.7
T4AA	1.4	0.9		1.0	1.0	0.9
FREE T3	3.1	3.6		3.5	2.8	2.6
TSH	0.15	0.27		0.58	0.13	0.46
QUANT. PLATELET	330	121		276	309	.
ANISOCYTOSIS	Slight	Slight		Slight	Slight	Slight

	Control/Antioxidant					
Animal ID	**1508A**	**1491B**	**1523B**	**1509U**	**1532S**	**1581S**
Date	2/9/00	3/21/00	3/21/00	2/9/00	2/9/00	3/21/00
Tests Performed						
AST (SGOT)	35	24	24	19	25	28
ALT (SGOT)	61	41	32	17	38	61
T. BILIRUBIN	0.3	0.2	0.1	0.2	0.3	0.1
ALK PHOS	246	187	91	134	389	72
GGT	1	5	3	2	1	6
TOTAL PROTEIN	6.3	6.0	6.0	6.3	6.2	5.4
ALBUMIN	3.3	3.4	3.4	3.4	3.1	3.4
GLOBULIN	3.0	2.6	2.6	2.9	3.1	2.0
A/G RATIO	1.1	1.3	1.3	1.2	1.0	1.7
CHOLESTEROL	154	228	198	286	344	191
BUN	13	16	11	14	14	10
CREATININE	0.5	1.2	0.6	0.5	0.5	0.5
BUN/CREAT	26	13	18	28	28	20
PHOSPORUS	3.6	3.0	5.6	3.8	2.7	3.1
CALCIUM	10.2	10.1	9.5	10.6	9.3	8.8
CA/PO4	2.8	3.4	1.7	2.8	3.4	2.8
GLUCOSE	83	90	82	87	92	84
AMYLASE	798	648	962	536	838	714
LIPASE	238	190	348	285	688	322
SODIUM	145	140	143	142	143	143
POTASSIUM	4.6	4.1	4.7	4.5	3.9	4.5
NA/K RATIO	32	34	30	32	37	32
CHLORIDE	111	107	110	111	112	107
CPK	104	139	118	109	270	174
TRIGLYCERIDE	38	103	56	59	71	79
OSMOLALITY- CALC	299	291	294	294	296	294
CORRECTED CA	10.4	10.2	9.6	10.7	9.7	8.9
MAGNESUIM	1.6	2.1	2.3	2.1	1.9	2.1
WBC	11.7	6.6	5.9	9.6	6.1	7.2
RBC	7.5	7.7	8.0	6.4	7.2	7.4
HGB	17.5	17.8	17.4	14.3	15.8	16.3
PCV	53	54	54	44	48	50
MCV	70	71	68	69	67	67
MCH	23.4	23.2	21.9	22.2	21.8	22.0
MCHC	33	33	32	33	33	33
NEUTROPHILS	75	79	63	71	72	73
ABSOLUTE	8775	5214	3717	6816	4392	5256
LYMPHOCYTES	15	15	27	18	20	22
ABSOLUTE	1755	990	1593	1728	1220	1584
MONOCYTES	9	6	10	6	7	5
ABSOLUTE	1053	396	590	576	427	360
EOSINOPHILS	.	.	.	5	.	.
ABSOLUTE	.	.	.	480	.	.
BASOPHILS	1	.	.	.	1	.
ABSOLUTE	117	.	.	.	61	.
PLATELET EST.	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate

Animal ID	1508A	1491B	1523B	1509U	1532S	1581S
Date	2/9/00	3/21/00	3/21/00	2/9/00	2/9/00	3/21/00
T3 (RIA)	117	140	105	108	130	108
T4 (RIA)	102	1.82	0.72	1.39	1.42	1.23
FREE T4 (RIA)	1.23	1.58	0.94	1.01	0.84	1.60
T3AA	0.9	0.9	1.0	1.0	1.6	0.8
T4AA	0.9	0.7	0.7	1.2	1.1	0.6
FREE T3	3.4	4.1	2.5	2.7	3.3	3.5
TSH	0.38	0.52	0.29	0.49	0.28	0.16
QUANT. PLATELET	435	180	308	336	357	386
ANISOCYTOSIS	Slight	Slight	Slight	Slight	Slight	Slight

	Enriched/Antioxidant					
Animal ID	**1541B**	**1585A**	**1521B**	**1502S**	**1542T**	**1581T**
Date	3/21/00	3/21/00	3/21/00	2/9/00	2/9/00	3/21/00
Tests Performed						
AST (SGOT)	27	40	35	36	22	25
ALT (SGOT)	29	39	36	57	19	38
T. BILIRUBIN	0.2	0.1	0.1	0.2	0.3	0.1
ALK PHOS	91	100	60	122	186	139
GGT	3	3	4	6	4	3
TOTAL PROTEIN	5.7	5.8	6.0	6.7	6.6	6.2
ALBUMIN	3.6	3.2	3.5	3.2	3.5	3.7
GLOBULIN	2.1	2.6	2.5	3.5	3.1	2.5
A/G RATIO	1.7	1.2	1.4	0.9	1.1	1.5
CHOLESTEROL	175	212	183	392	222	279
BUN	11	15	10	13	9	8
CREATININE	0.6	0.7	0.5	0.5	0.5	0.5
BUN/CREAT	18	21	20	26	18	16
PHOSPORUS	4.2	4.0	4.6	5.2	2.9	4.9
CALCIUM	9.3	9.6	9.5	10.6	10.1	10.3
CA/PO4	2.2	2.4	2.1	2.0	3.5	2.1
GLUCOSE	77	77	74	73	75	92
AMYLASE	812	760	849	595	661	439
LIPASE	202	120	57	221	573	438
SODIUM	141	142	141	140	142	143
POTASSIUM	4.6	4.4	4.4	4.4	4.3	4.2
NA/K RATIO	31	32	32	32	33	34
CHLORIDE	106	109	107	105	110	103
CPK	131	252	184	162	124	106
TRIGLYCERIDE	120	74	61	270	87	162
OSMOLALITY- CALC	290	294	290	289	291	294
CORRECTED CA	.	9.9	.	10.9	.	.
MAGNESUIM	1.8	2.0	2.2	2.3	2.1	2.4
WBC	6.3	8.3	4.7	10.3	4.6	5.3
RBC	6.1	6.9	6.3	6.5	8.8	8.4
HGB	14.9	15.5	14.5	14.7	17.1	18.1
PCV	45	48	44	45	53	56
MCV	74	70	70	70	61	67
MCH	24.4	22.5	23.0	22.6	19.5	21.6
MCHC	33	32	33	32	32	32
NEUTROPHILS	74	70	70	76	74	75
ABSOLUTE	4662	5810	3290	7828	3404	3975
LYMPHOCYTES	17	17	21	15	20	18
ABSOLUTE	1071	1411	987	1545	920	954
MONOCYTES	8	12	6	9	6	7
ABSOLUTE	504	996	282	927	276	371
EOSINOPHILS	.	.	2	.	.	.
ABSOLUTE	.	.	94	.	.	.
BASOPHILS	1	1	1	.	.	.
ABSOLUTE	63	83	47	.	.	.
PLATELET EST.	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate

Animal ID	1541B	1585A	1521B	1502S	1542T	1581T
Date	3/21/00	3/21/00	3/21/00	2/9/00	2/9/00	3/21/00
T3 (RIA)	104	100	113	139	94	134
T4 (RIA)	1.18	0.99	0.98	0.70	1.35	1.38
FREE T4 (RIA)	1.23	1.24	1.19	0.70	1.09	1.71
T3AA	0.8	1.1	1.0	2.0	1.0	0.9
T4AA	0.7	0.8	0.8	1.1	1.2	0.7
FREE T3	2.9	3.2	3.0	3.4	2.4	3.7
TSH	1.16	0.43	1.07	3.55	0.32	0.49
QUANT. PLATELET	296	323	347	420	218	172
ANISOCYTOSIS	Slight	Slight	Slight	Slight	Slight	Slight

Appendix B. Abstract

Oxidative Stress, β-Amyloid and Cognitive Function in the Aged Canine Brain

E. Head, J. Liu, B. N. Ames, B. A. Muggenburg, N. W. Milgram, and C. W. Cotman

Several studies report evidence of oxidative stress being increased in aged human brain. We examined the extent of lipid peroxidation in the aged canine, which we have suggested to be a model of human brain aging. Eighteen dogs ranging in age from 2-15 years were tested for size discrimination learning and subsequently the extent of β-amyloid (Aβ) deposition was measured in the prefrontal cortex using immunostained 4% paraformaldehyde-fixed sections. Oxidative damage to lipids was measured in serum and in frozen blocks from the contralateral prefrontal cortex using a biochemical assay for malondialdehyde (MDA) using GC-MS methodology. MDA levels were significantly elevated with aging ($F(1,17)=12.144$ $p<.003$). In addition, MDA levels were a significant predictor of Aβ deposition in the prefrontal cortex ($r=.70$ $p<.001$, $n=13$) even when the effects of age were partialled out ($r=.5041$, $p<.039$ $n=13$). Further, MDA levels were significantly correlated with cognitive function; higher error scores were associated with increased levels of MDA ($r=.534$ $p<.04$). Finally, serum levels of MDA predicted brain levels of MDA ($r=.494$ $p<.037$) and thus serum assays may serve as a measure of brain oxidative stress. These results suggest that like the human, dogs show evidence of oxidative stress in the brain that is associated with age, the extent of Aβ deposition and with cognitive function.

Supported by NIA AG12694 and USAMRMC.

Appendix C. Abstract

Visual Object Recognition Memory in Beagle Dogs: Effects of Processing Time and Age

C. J. Ikeda-Douglas, H. Callahan, E. Head, C. Cotman, J. Araujo, A. Chan, J. Estrada, B. Muggenberg, S. Zicker, N. W. Milgram, and W. Mackay

We have previously reported that dogs have considerable difficulty in acquiring a visual based delayed-non-matching-to-sample task (DNMS), but acquisition can be improved by introducing a pause during the presentation of the sample. In this study, young (2-4 yrs) and aged (>9 yrs) dogs were given up to 60 acquisition sessions on the DNMS task. A large number of both young and old dogs were unable to pass the preset criterion. Age did not affect rate of learning, among those that did acquire the task. The dogs that passed at 10 second delay were given an additional 40 training sessions, using a maximal memory paradigm involving testing at increasingly longer delays. Marked age differences were observed, with young dogs achieving significantly longer delays than old. We also examined the effects of object presentation time by varying the length of the pause upon presentation of the sample. As expected, performance was directly related to duration of presentation time. These results indicate that age is not a critical factor in learning a DNMS task in dogs, but can profoundly affect memory capacity. Other factors affecting acquisition are previous background and allowable processing time.

Supported by: NIA AG12694 and USAMRMC.

Appendix D. Abstract

Landmark Discrimination Learning in Aged Dogs is Improved by Treatment with an Antioxidant Enriched Diet

N. W. Milgram, J. Estrada, C. Ikeda-Douglas, J. Castillo, E. Head, C.W. Cotman, H. Murphey, D. Holowachuk, B. Muggenburg, and S. Zicker

Reactive oxygen species, which are byproducts of cellular metabolism, are potentially critically important contributors to degenerative neural changes that accompany aging. We fed a canine diet consisting of a broad spectrum of antioxidants and tested the effects on age-dependent cognitive deterioration in beagle dogs. Young and aged beagle dogs were each divided into groups receiving either control or antioxidant-enriched diets. The dogs were placed on the diet for either 0 or 5 weeks, and were then tested on a series of discrimination problems, all of which required the animals to respond selectively to the object closest to an external landmark. The aged animals on the enriched diet learned all of the tasks more rapidly than did the aged animals on the control diet. More consistent improvement was seen initially in the animals given 5 weeks of diet before testing. The young animals, by contrast, showed no effect of diet. These results both further support a free radical model of age-dependent neurodegeneration and indicate that short-term administration of antioxidants can partially reverse the deleterious effects of aging on cognition.

Supported by: NIA and USAMRMC.

Appendix E. Abstract Selection for Press Conference in New Orleans

Dear Dr. Milgram,

We are in the process of pulling together potential press conferences for the 2000 annual meeting. A request was mailed to Dr. Zicker in July, but we have not heard back from him. This e-mail is to see if you will be attending the meeting in New Orleans and if you would be interested in presenting in a proposed press conference about Diet & The Brain.

Below please find a description of the proposed press conference, a memorandum requesting lay language summaries for the paper "LANDMARK DISCRIMINATION LEARNING IN AGED DOGS IS IMPROVED BY TREATMENT WITH AN ANTIOXIDANT ENRICHED DIET," and a copy of your abstract. If you could let me know as soon as possible if you will be willing to prepare a summary, it would be most appreciated.

Many thanks,
Mary C. McComb

Mary C. McComb
Public Information Assistant
Society for Neuroscience
11 Dupont Circle, Suite 500
Washington, DC 20036
Phone: (202) 462-6688
E-mail: mary@sfn.org

DESCRIPTION:

Diet & the Brain. Abstracts. G. Spiller on breakfast consumption enhancing long term declarative memory in seniors (3903); D. Dansereau on memory impairments in aspartame users (9840); M. Mattson on dietary restriction increasing neurogenesis in adult rat (6919); N. Milgram on learning in dogs is improved by antioxidant enriched diet (3613); and B. Hoebel on sugar addiction (7586). Moderated by B. Hoebel.

MEMORANDUM

FROM: Joe Carey, Public Information Director

RE: 2000 Annual Meeting Press Conference, Request for Lay Language Summary

DATE: July 2000

Public awareness of neuroscience research is vitally important, especially its great potential for understanding the brain and nervous system, and for treating brain disorders. The Society fulfills its obligation to inform the public and to insure continued support for neuroscience research through Annual Meeting press conferences and news releases on popular topics.

This year, your abstract is being considered by the Public Information Committee for inclusion in a press conference at the Annual Meeting. If selected for a press conference, your material will be written up as part of a news release which will be distributed to hundreds of members of the national and international media. During the press conference itself, a panel of three to four knowledgeable scientists in the same field will provide perspective by presenting their findings alongside yours in a 10 to 15 minute presentation.

Appendix F. Abstract

A Novel Spatial Learning and Memory Task: The 3-Component Delayed Non-Matching-to-Position Task

P. Nippak, A. Chan, B. Adams, C. Ikeda-Douglas, E. Head, C. Cotman, B. Muggenberg, S. Zicker and N. W. Milgram

The DNMP task, as typically used begins with a sample presentation, followed by a delay and ends with a choice between the two spatial locations – sample and nonmatch. A correct choice is a response to the nonmatch position. As there are only two positions, the exact location of the correct response is determined by the sample. We have developed a novel two-choice DNMP task that uses three positions, the 3-component DNMP (3cDNMP). In this version, the correct response cannot be anticipated from the sample. Subtests are created from the three sample positions. This allows for a qualitative analysis of cognitive-behavioural processes. Three groups of dogs were tested on the 3cDNMP: two naïve groups, young and aged, who had no prior DNMP training, and an aged experienced group who had 5 months of typical DNMP training. Three different learning strategies were observed: employment of a nonmatch rule – most proficient, differential acquisition of subtests, and a positional strategy – unsuccessful. The young and aged employed successful strategies, however, the majority of aged employed positional strategies and failed to acquire the task. Performance varied as a function of subtest, with the majority of errors committed when the sample is in the centre location. The inability to anticipate the correct response makes the 3cDNMP less vulnerable to mediating strategies (e.g., orientation) and more difficult to learn and perform accurately on.

Supported by: NSERC and USAMRMC.